Effect of L-arginine on renal blood flow in normal subjects and patients with hypoxic chronic obstructive pulmonary disease

T Q Howes, S E J Keilty, V L Maskrey, C R Deane, S V Baudouin, J Moxham

Abstract

Background – L-arginine is the precursor of endothelium derived nitric oxide (NO) and increasing the available substrate may increase the production of NO. This has been shown by local infusion in peripheral vascular beds but there are few studies of the effects during systemic infusion. Renal vasoconstriction is known to be important in the pathogenesis of cor pulmonale in patients with hypoxic chronic obstructive pulmonary disease (COPD). The effects of a systemic infusion of L-arginine on renal and aortic haemodynamics were therefore investigated in normal subjects and in patients with hypoxic COPD.

Methods – Ten normal volunteers were recruited from the research staff of King's College Hospital. Six patients with COPD and hypoxia (arterial oxygen tension (PaO_2) <8.5 kPa) were recruited from the thoracic medicine outpatient clinic at King's College Hospital and five age and sex matched normal subjects were recruited from a group of normal subjects recruited from the database of the Department of Health Care for the Elderly as volunteers for medical research. There was no history of renal, cardiac, or hepatic disease. Baseline values of time averaged mean of the maximum instantaneous velocity (Tamx) and maximum velocity (Vmax) of blood flow in intrarenal arteries were obtained using colour flow Doppler ultrasound. Using the same technique, Vmax was obtained from the abdominal aorta just distal to the xiphisternum before and after infusion of L-arginine via a large peripheral vein (20 g in 100 ml sterile water over 30 minutes).

Results – In normal subjects L-arginine increased blood velocity in the intrarenal vessels from a mean of 0.22 m/s to 0.26 m/s, an increase of 19.8% . There was no effect on arterial blood pressure, heart rate, or aortic blood velocity. L-arginine had no effect on intrarenal or aortic blood velocity in patients with hypoxic COPD. In age matched controls L-arginine increased blood velocity in the intrarenal vessels from a mean of 0.20 m/s to 0.26 m/s, an increase of 36.8% . There was no effect on arterial blood pressure, heart rate, or aortic blood velocity.

Conclusions – L-arginine, at the doses administered, increased renal blood flow, as assessed by renal arterial velocity. This

effect was not seen in patients with hypoxic COPD but was present in age matched controls. This suggests that the abnormal renal vascular control seen in hypoxic patients with COPD may reflect a disturbance of the L-arginine/nitric oxide pathway.

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Keywords: L-arginine, renal blood flow, chronic obstructive pulmonary disease, colour flow Doppler ultrasound.

Reduced renal blood flow is important in the pathogenesis of congestive cardiac failure^{1,2} and cor pulmonale.³ The mechanisms of the fall in renal perfusion in these conditions remains elusive.⁴ Cardiac output is often relatively well preserved⁵ and it has been suggested that the reduction in renal flow represents a rise in renovascular resistance.

Colour flow Doppler ultrasound can detect changes in renal blood flow¹ and has been used to show that oxygen is a renal vasodilator in cor pulmonale in patients who are hypoxaemic and normocapnic.⁶ In this work flow velocity rose in renal vessels by 25% with oxygen. This rise was comparable to changes seen in these subjects with low dose dopamine infusions. In hypercapnic patients oxygen or low dose dopamine do not dilate the renal vasculature.⁷ It is possible that the failure to vasodilate reflects progressive endothelial damage of the renal vasculature secondary to prolonged hypoxaemia. Endothelium derived relaxing factor (EDRF) is well established as an important regulator of vascular tone^{8,9} and is now known to be nitric oxide (NO). Prolonged hypoxaemia may cause impairment of production or release of EDRF from the endothelium; initially it is reversible with oxygen therapy, but becomes irreversible as hypoxaemia becomes more severe and patients develop CO_2 retention. In the human umbilical vein endothelial subculture hypoxia and the nitric oxide synthase inhibitor, N-w-nitro-L-arginine have additive effects on the production of endothelin (a potent vasoconstrictor), but both can be reversed by exogenous NO.¹⁰ In the pulmonary circulation of the fetal lamb oxygen is able to enhance NO production¹¹ and this effect has also been shown in isolated human mammary artery.¹²

NO is formed from L-arginine in endothelial tissue¹³ by the action of NO synthase, now known to be a flavoprotein.¹⁴ There is evidence that exogenous L-arginine releases nitric oxide in vitro in the presence of this enzyme.¹⁵ A

Department of
Thoracic Medicine
T Q Howes
S E J Keilty
J Moxham

Department of Health
Care for the Elderly
V L Maskrey

Department of
Medical Engineering
and Physics
C R Deane

King's College School
of Medicine and
Dentistry,
London, UK

Department of
Intensive Care
Medicine,
St James' Hospital,
Leeds, UK
S V Baudouin

Correspondence to:
Dr T Q Howes,
Colchester General Hospital,
Colchester, Essex CO4 5JL,
UK.

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study in man has shown vasodilatation at a dose of 30 g over half an hour with a concomitant rise in urinary nitrite and plasma cyclic GMP and L-citrulline levels,¹⁶ but another study showed no effect on systemic haemodynamics at a slightly higher dose.¹⁷ Other studies have shown a small fall in mean arterial pressure and a rise in renal perfusion as measured by clearance of PAH.¹⁸ Studies in dogs have shown that L-arginine is a renal vasodilator without any effect on cardiac output.¹⁹

We wished to investigate the effects of L-arginine on renal perfusion in normal man and in subjects with hypoxic chronic obstructive pulmonary disease (COPD) using colour flow Doppler. We hypothesised that, if the renal vasodilator effects of oxygen were mediated via the NO pathway, then L-arginine would also have this effect. We further suggest that, if the underlying mechanism lies with this pathway, then hypoxia would blunt the effects of L-arginine. Patients with hypercapnia were excluded because we have previously shown that their renal vascular responses are unresponsive to conventional dilators of the renal arterial tree such as low dose dopamine⁷; any failure to respond to L-arginine infusion in these subjects may be related to this generalised failure to respond to any vasodilator. Renal haemodynamics and aortic blood velocity (as an index of cardiac output) were measured simultaneously in the normal subjects. Subjects with hypoxic COPD were compared with a group of age and sex matched subjects. This last group were investigated in a double blind fashion using an "active" placebo. The placebo was an identical looking infusion of hypertonic 3N saline offering an equivalent osmotic load to the L-arginine infusion.

Methods

Colour flow Doppler ultrasound (Acuson 128, Acuson Inc, Mountain View, California, USA) was used to measure changes in blood flow. This technique has been validated as a measure of renal blood flow changes⁷ and as a measure of changes in cardiac output.^{20,21}

Ten normal volunteers recruited from hospital staff were studied. They gave no history of any condition affecting the kidneys, heart, or liver, and no history of serious illness. During the studies heart rate and blood pressure were monitored automatically and non-invasively (Critikon Dinamap 1846 SX). After a 30 minute rest, readings were taken independently by two observers (TH and CD) from selected intrarenal vessels of the right kidney using the Doppler scanner, with a 3.5 MHz phased array transducer scanning in a parasagittal plane over the liver. The two observers selected vessels that could be reliably identified but not necessarily the same vessel. In previous work there was an interobserver error of around 6%.⁶ When individual vessels could be reliably found in which the angle between the ultrasound beam and the blood flow was less than 30°, velocity measurements were obtained. Changes were calculated from the arithmetic mean of steady state velocity readings obtained in the

baseline period before and washout periods after the infusions. This is in accordance with previous validation of the Doppler technique in measuring changes in renal flow.⁷ In addition, the same transducer was used in the midline, immediately distal to the xiphisternum, to obtain velocity measurements from the aorta. The ultrasound probe was angled towards the subject's head to minimise the angle between the ultrasound beam and the vessel. It was technically possible to obtain readings using this technique in only three of the patients with COPD. In view of this no attempt was made to measure aortic flow in the age matched controls.

L-Arginine was prepared in advance by the pharmacy department of Guy's Hospital, London, UK. Using a bacterial filter commercially available L-arginine (Sigma Pharmaceuticals, Poole, UK) was dissolved in sterile water, stored in single use vials, and kept at 4°C until use. Each subject was infused with 20 g L-arginine in 100 ml over a 30 minute period (3.1 mmol/min). Doppler studies were performed after the infusion had been running for five minutes over a period of 30 minutes. A few minutes after the infusion had stopped the Doppler scanner was employed for further studies of the renal vessels and aorta for 15–20 minutes.

In further studies six patients with COPD were recruited from the thoracic medicine outpatient clinic. Criteria for entry were (1) longstanding COPD with a forced expiratory volume in one second (FEV₁) of less than 1.5 l and peripheral oedema during a previous admission with an acute exacerbation of COPD, and (2) arterial oxygen tension (Pao₂) of less than 8.5 kPa whilst breathing air. The patients were compared with a group of normal volunteers matched for age and sex who were recruited from a database of normal subjects at the Department of Health Care for the Elderly of King's College Hospital. Solutions of L-arginine and 3N saline were infused in a double blind fashion. This solution of saline is a comparable osmotic load to the dose of L-arginine. The infusions of L-arginine were the same as those used in the normal subjects and those with hypoxic COPD.

STATISTICAL ANALYSIS

Doppler ultrasound velocity measurements of the renal arteries may not be normally distributed so non-parametric tests were used to analyse the data. Using an Apple MacIntosh computer running Instat 2.01 software Wilcoxon's signed rank test was used to test pairs of results in the same patient with or without L-arginine and versus placebo or, when applicable, 3N saline.

Results

NORMAL SUBJECTS

Good quality intrarenal artery Doppler measurements were possible in all normal subjects and adequate aortic measurements were possible in five. The infusion of L-arginine did not

Table 1 Time adjusted mean velocity of maximum flow (T_{amx}) in selected intrarenal vessels (m/s) and maximal systolic flow (V_{max}) in the aorta in normal subjects

Subject no.	Baseline	L-Arginine	Washout	Increase (%)
Renal flow:				
1	0.2	0.21	0.16	17
2	0.16	0.2	0.16	25
3	0.18	0.24	0.18	33
4	0.22	0.24	0.2	14
5	0.22	0.3	0.22	36
6	0.18	0.21	0.19	14
7	0.2	0.23	0.23	15
8	0.34	0.46	0.36	31
9	0.23	0.25	0.21	14
10	0.25	0.26	0.24	8
Mean				19.8*
Difference (%)				
Aortic flow:				
1	0.94	1.05	0.97	+9
2	1.03	1.08	1.06	+2
3	0.74	0.76	0.79	-1
6	0.77	0.78	0.81	-1
7	0.98	0.99	1.02	-1

Each value is the mean of five measurements made over a 15–30 minute period. Differences were taken as changes from a baseline flow calculated from the arithmetic mean of baseline and washout flow.

* $p < 0.05$.

Table 2 Changes in renal flow in patients with hypoxic COPD and age matched controls expressed as time adjusted mean velocity of the maximum flow (T_{amx}) of the blood in selected intrarenal vessels (m/s)

Subject no.	Baseline	L-Arginine	3N Saline	Increase with L-arginine (%)
Age matched controls:				
1	0.2	0.23	0.19	15
2	0.18	0.24	0.19	30
3	0.31	0.41	0.30	34
4	0.23	0.26	0.26	6
5	0.18	0.24	0.16	41
6	0.10	0.20	0.13	73
Mean				36.8*
Subjects with hypoxic COPD:				
1	0.21	0.21	—	-1.9
2	0.21	0.20	—	-0.5
3	0.21	0.19	—	-13
4	0.09	0.10	—	+11
5	0.25	0.25	—	+0
6	0.09	0.09	—	+3.4

Each value is the mean of five results taken over a 15–30 minute period. Differences were taken as changes from a baseline flow calculated from the arithmetic mean of baseline and washout flow.

* $p < 0.05$.

have any effect on blood pressure, heart rate, or aortic blood velocity. There was, however, a significant increase in intrarenal arterial blood velocity of 19.8% ($p = 0.002$). Mean (SE) baseline renal blood velocity was 0.22 (0.05) m/s which rose to 0.26 (0.08) m/s with L-arginine. Mean (SE) aortic blood velocity was 0.89 (0.13) m/s during the baseline period, 0.93 (0.15) m/s during L-arginine infusion, and 0.93 (0.13) during the washout period (table 1).

SUBJECTS WITH HYPOXIC COPD AND AGE MATCHED CONTROLS

The subjects with COPD had a mean age of 65 years (range 56–75) and impaired pulmonary function (mean FEV_1 0.65 l (range 0.6–1.0) and forced vital capacity (FVC) 1.91 (1.55–2.0)). They were all hypoxaemic but normocapnic (mean P_{aO_2} 7.9 kPa (range 6.5–8.5), P_{aCO_2} 5.3 kPa (range 4.6–6.1)). Good quality intrarenal artery Doppler measurements were possible in all six subjects with COPD and the six age matched controls, and adequate aortic measurements were possible in three of the

subjects with COPD; aortic measurements were not performed in the age matched group. The infusion of L-arginine did not have any effect on blood pressure or heart rate. In addition, the COPD group showed no change in renal haemodynamics: mean (SE) renal blood velocity was 0.176 (0.07) m/s during the baseline period and 0.173 (0.06) m/s during L-arginine infusion. The age matched controls had similar haemodynamic responses to the younger group. Infusion of 100 ml hypertonic 3N saline had no effect on the renal haemodynamics in this group. Renal blood velocity was 0.20 (0.07) m/s during the baseline period, 0.26 (0.07) m/s with L-arginine (36.8% increase, $p = 0.002$), and 0.20 m/s during the infusion of hypertonic 3N saline (table 2).

Discussion

In the cells of the arterial endothelium NO is synthesised from the nitrogen moiety of L-arginine.²² This reaction is catalysed by nitric oxide synthase. In vascular smooth muscle the resulting NO is responsible for vasodilatation.^{13,23} Continuous NO-dependent vasodilator tone is present and this may be enhanced with L-arginine and reduced with blockers such as N^G -monomethyl-L-arginine (L-NMMA).⁹ This basal NO production is responsible at least in part for the maintenance of adequate basal flow in vascular beds. There is much evidence that hypoxia induces vasodilatation in many vascular beds.^{13,24} However, we have previously shown that reversing the hypoxia causes renal vasodilatation in hypoxic patients with COPD.^{6,7} In the kidney NO has been shown to be an important modulator of vascular tone,²⁵ opposing the effects of angiotensin II and endothelin. If hypoxia were to lead to a reduction in NO production, the resulting unopposed actions of these vasoactive substances might be more pronounced in the kidney than in other organs because the renal vasculature is more sensitive to vasoconstrictors.²⁶ If the renal vasodilator endothelial production of NO is limited by the availability of substrate, then stimulating the NO pathway in the kidney with, for example, exogenous L-arginine should increase renal blood flow. Other workers have observed concomitant systemic and renal perfusion changes in man during L-arginine infusion.¹⁸ They demonstrated a rise in renal blood flow using a clearance technique and simultaneous changes in blood pressure and pulse rate consistent with systemic vasodilatation during an infusion of L-arginine at 30 g over 30 minutes. Using colour flow Doppler ultrasound we have shown that renal blood flow is increased in normal humans during an infusion of L-arginine of 20 g over 30 minutes in the absence of changes in cardiac output. This is in agreement with Murakami *et al* who have investigated renal blood flow and cardiac output in dogs and shown similar results.¹⁹ They also performed simultaneous assays of plasma renin activity and atrial natriuretic factor which did not change with L-arginine. Denervation of the kidney and atropine administration reduced the

increase in renal blood flow in these animals. However, other studies have found no change in systemic arterial pressure during infusions of L-arginine.¹⁷ In our study we have shown that the increase in renal blood flow seen with infusions of L-arginine in humans is independent of changes in systemic blood flow. This suggests that L-arginine acts on the renal vasculature to produce vasodilatation. This may be due to an increase in NO production.

In the group of patients with hypoxic COPD L-arginine had no effect on renal blood flow. Defective endothelial function has been demonstrated in forearm arteries in patients with heart failure secondary to ischaemic heart disease,²⁷ and our results suggest that defective endothelial function is present in the renal vessels of patients with cor pulmonale. This is in contrast to patients with congestive heart failure secondary to ischaemic heart failure whose renal blood flow is relatively preserved.²⁸

In a previous study we have shown that oxygen is a potent renal vasodilator in hypoxic patients with COPD.⁶⁷ A possible mechanism of this effect is that oxygen, which is necessary for the production of nitric oxide, is deficient in the endothelium of these patients and therefore NO dependent vasodilator tone is reduced causing vasoconstriction. Alternatively, oxygen concentrations might be necessary at a high level to synthesise nitric oxide by a different mechanism (an example might be by influencing the availability of ATP). Finally, this effect of oxygen might be via a different mechanism. These hypotheses might be addressed by assessing renal blood flow with and without oxygen therapy in the presence and absence of infused L-arginine. However, in this study we have shown a disturbance in the L-arginine/nitric oxide pathway in hypoxic patients with COPD. This is evidence that the derangement in renovascular control in patients with COPD and cor pulmonale, and the renal vasodilatation seen with oxygen therapy in these patients, may be caused, at least in part, by an abnormality of the nitric oxide pathway. Further work, as outlined above, will be needed to clarify this.

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